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14. ABSTRACT

In this project I wanted to investigate how cellular patterns of histone modifications differ in breast cancer depending on the stage of the disease, and whether this cellular heterogeneity could be related to the clinical outcome of the cancer patient. I used antibodies specific to histone modifications and variants to probe the epigenome of breast cancer progression. I compared the levels of the histone modifications and variants in several breast cancer cell lines (ErbB2 Amplified, ER/PR positive and Triple negative) comparing it to the non-tumorigenic cells. Initial results show that the levels of some of the histone modifications and variants correlate with the hormone receptor status of these cells. Further work in a larger panel of the cell lines or patients using immunohistochemistry will be crucial in determining the significance of these findings.

15. SUBJECT TERMS

Histone modifications, Histone Variants, Epigenetics

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Overall summary of the project period 1/13/11-6/30/11

In the past year, with the support of the DOD funding I have procured several breast cancer cell lines and performed preliminary analysis examining the changes in histone modifications and variants during breast cancer progression. I have attended scientific meetings using the funding provided by the DOD. These meetings were an important part of my training plan and not only give me an opportunity to present my ongoing work but also foster collaboration between institutions.

Introduction

Cancer is a disease of both genetic and epigenetic changes. Epigenetics may be defined as the study of any potentially stable, and ideally, heritable change in gene expression or cellular phenotype that occurs without changes in DNA (Goldberg et al., 2007). Recently, the role of epigenetics in cancer has become increasingly apparent. While aberrant DNA methylation in cancer is well studied, a growing body of evidence suggests that other chromatin-mediated processes are linked to the development and progression of numerous cancers, including breast cancer (Wang et al., 2007; Mulero-Navarro and Esteller, 2008; Elsheikh et al., 2009). Recent reports have revealed that global histone PTM patterns on the H3 tail, including lower levels of H3K4me2 and H3K18ac, predict a higher risk of prostate cancer recurrence; these PTM profiles also predict clinical outcome in both lung and kidney cancer (Seligson et al., 2005; Seligson et al., 2008). Increased levels of the histone variant H2A.Z are significantly associated with lymph node metastasis and decreased breast cancer survival, and independently increase the prognostic power of biomarkers currently in clinical use for breast cancer (Hua et al., 2008; Svotelis et al., 2010). My recent work focusing on malignant melanoma demonstrates that global changes of histone PTMs and histone variants strikingly correlate with disease progression, in particular, the histone variant macroH2A. Together, these finding are instrumental in demonstrating that numerous histone-related changes occur in cancer. *However, a thorough and high-throughput investigation of the epigenetic changes that occur during breast cancer progression has yet to be carried out an important aim of my current proposal.*

Body

In this grant I had proposed to examine the epigenetic changes that take place during the development of breast cancer, the second most common cancer among women in the US. In order to achieve this aim, I had proposed to compare global histone PTM and histone variant profiles in a panel of human cells lines. I procured several cell lines from ATCC that represent different subtypes of breast cancer and have been well characterized in terms of oncogenic function. These include non-tumorigenic (185B5 and MCF-10A), tumorigenic, but non-metastatic and ErbB2 amplified (BT474), tumorigenic, non-metastatic ER/PR positive MCF-7 and T47D), and triple negative (MM157 and MM231) cells. In my statement of work (SOW) I had proposed to first

perform quantitative mass spectrometry analysis of these cell lines to compare the levels of histone modifications and variants in an unbiased manner. However I decided to first perform a proof of principle experiment to test if the panel of cell lines can be used to represent the various subtypes of breast cancer. I extracted chromatin from the above-mentioned cells and immunoblotted for several histone modifications and variants. For the histone modifications I initially focused on methylation and acetylation marks of histone H3 and H4 since they have been linked to gene activation and repression while for the histone variants I focused on the histone variant macroH2A, which is the focus of research in our lab. Of note I have recently successfully used a similar approach to probe the epigenome during melanoma progression. In particular, I observed a remarkable loss of the histone variant isoforms macroH2A1 and macroH2A2 in both human and mouse melanoma cell lines (Kapoor et al., 2010). I subsequently applied this observation to three different cohorts with combined >150 human tissue samples, consisting of benign nevi, primary melanomas and melanoma metastases. Using immunohistochemistry (IHC) studies, I observed the loss of macroH2A staining in over 70% of primary and metastatic melanoma tissues, while almost all nevi stain positively for macroH2A (Kapoor et al., 2010).

Key Research Accomplishments

Using a well-defined series of breast cancer cell lines I aimed to produce a 'nucleosome level signature' associated with different subtypes of breast cancer. I performed limited analysis of chromatin from the breast cancer cell lines by means of the large histone antibody collection in the laboratory. I demonstrate that changes occur for some of histone modifications in distinct breast cancer subtypes (Figure 1). For example levels of H3K9ac (Histone 3 lysine 9 acetylation) increase in all breast cancer subtypes compared to the non-tumorigenic counterparts. Similarly levels of H4K20me3 increase in ER/PR positive and triple negative breast cancer cell lines compared to the non-tumorigenic controls while the levels of H4K20me1 decrease specifically in the ER/PR positive cell lines.

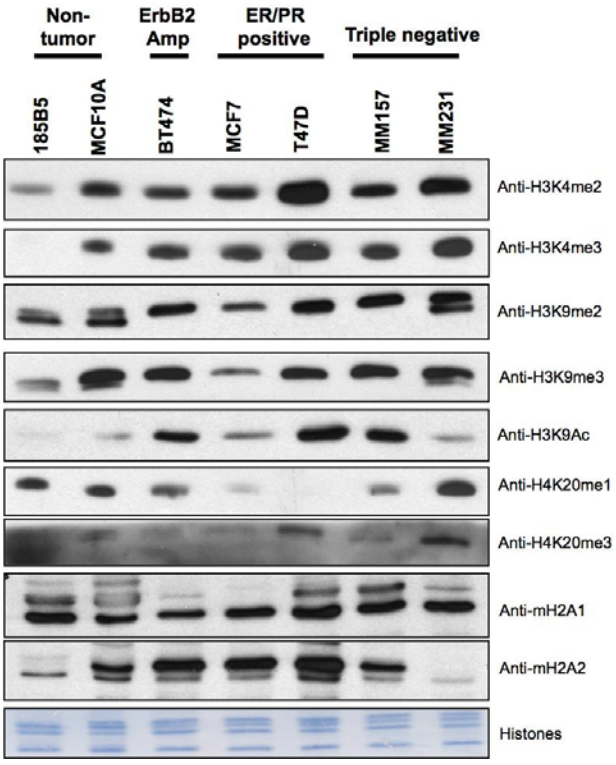

Figure 1. Breast cancer cells probed for histone modifications and histone variants; core histones used for loading

For the histone variant macroH2A unlike melanoma where we saw a dramatic loss of it we only see a downregulation of macroH2A2 in the triple negative breast cancer cell lines while the levels of macroH2A1 remain same.

Reportable Outcomes

In the limited funding period I was able to extract chromatin from a panel of breast cancer cell lines and characterize differences in histone modifications and variants in them. While I do observe differences in some histone modifications (such as H3K9ac, H4K20me3 and H4K20me1) the clinical significance of these changes and there potential to be used as biomarkers remains to be tested. However, I also recognize that I am not specifically addressing histone PTM or variant subcellular localization changes or gene-specific changes using this methodology.

Conclusion

Breast cancer is the second most common cancer diagnosed among women in the US, and is the second leading cause of cancer death in women after lung cancer. Like other cancers, it results from stepwise genetic alterations of normal host cells. However, it is becoming widely accepted that epigenetic alterations are universally present in human malignancies and that breast cancer is as much a disease of epigenetics alterations as it is of genetics. My work in malignant melanoma has demonstrated that epigenetic changes clearly take place during the progression of this highly metastatic and intractable disease. I very much appreciate that the DOD gave me to apply similar studies to breast cancer, which affects 1 in 8 women. While the genetics of breast cancer are well characterized, the epigenetic factors related to histone biology remain elusive. My long-term goal is to shed light on these changes and identify new players that represent potential targets for epigenetic therapies.

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